PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ING10692PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/10118	International filing date *(day/month/year)* 11.09.2003	Priority date *(day/month/year)* 13.09.2002
International Patent Classification (IPC) or both national classification and IPC A01K67/027		
Applicant INGENIUM PHARMACEUTICALS AG, et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08.04.2004	16.03.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Brouns, G Telephone No. +31 70 340-3789

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/10118

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-192 as originally filed

Claims, Numbers

1-185 as originally filed

Drawings, Sheets

1/29-29/29 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 79-94,96-100

   because:

   ☒ the said international application, or the said claims Nos. 79-94,96-100 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☒ all parts.

☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	27,36,38,39,60,70,74,76,77,122-185
	No:	Claims	1-26,28-35,37,40-59,61-69,71-73,75,78-121
Inventive step (IS)	Yes:	Claims	27,36,38
	No:	Claims	1-26,28-35,37,39-185
Industrial applicability (IA)	Yes:	Claims	1-78,95,101-185
	No:	Claims	79-94,96-100 (?)

2. Citations and explanations

see separate sheet

The present application relates to a mouse model for motor neuron disease with a ethyl-nitrosourea (ENU) induced point mutation in position Y1055C of the murine cytoplasmic dynein heavy chain1 protein. Furthermore, the full length polypeptide and polynucleotide sequence of the human cytoplasmic dynein heavy chain1 is provided.
The protein, antibodies, pharmaceutical compositions comprising said polypeptide, nucleic acid sequences, vectors, host cell, antisense nucleic acid, ribozyme, medicament comprising said polypeptide, a process for making said polypeptides, methods of preventing and diagnosing, a database, a method of gene delivery, a non-human animal model expressing said modified polypeptide, methods of identifying protein or nucleic acid markers indicative for the presence of a mutation in said polypeptide or polynucleotide, oligonucleotide for identifying a mutation in said polypeptide, kits comprising said oligonucleotides, solid support comprising said oligonucleotides and use of said kit or solid support are claimed.

1) Reference is made to the following documents:

D1: FAN JUAN ET AL: "Antibodies to cytoplasmic dynein heavy chain map the surface and inhibit motility" JOURNAL OF MOLECULAR BIOLOGY, vol. 307, no. 5, 13 April 2001 (2001-04-13), pages 1317-1327,

D2: TYNAN SHARON H ET AL: "Distinct but overlapping sites within the cytoplasmic dynein heavy chain for dimerization and for intermediate chain and light intermediate chain binding" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 42, 20 October 2000 (2000-10-20), pages 32769-32774

D3: BYERS H RANDOLPH ET AL: "Role of cytoplasmic dynein in melanosome transport in human melanocytes" JOURNAL OF INVESTIGATIVE DERMATOLOGY, vol. 114, no. 5, May 2000 (2000-05), pages 990-997

D4: DE ANGELIS MARTIN HRABE ET AL: "Genome-wide, large-scale production of mutant mice by ENU mutagenesis" NATURE GENETICS, vol. 25, no. 4, August 2000 (2000-08), pages 444-447

D5: HAFEZPARAST MAJID ET AL: "Mutations in dynein link motor neuron degeneration to defects in retrograde transport." SCIENCE (WASHINGTON D C), vol. 300, no. 5620, 2 May 2003 (2003-05-02), pages 808-812

D6: DATABASE EMBL EBI; 28 July 2000 (2000-07-28), SASAKI S ET AL: "Mus musculus cytoplasmic dynein heavy chain mRNA, complete CDS." XP002292030 Database accession no. AY004877

D7: NAGASE T ET AL: "Prediction of the coding sequences of unidentified human genes VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro" DNA RESEARCH, UNIVERSAL ACADEMY PRESS, JP, vol. 4, no. 2, 1997, pages 141-150

D8: LAMONTE BERNADETTE H ET AL: "Disruption of dynein/dynactin inhibits axonal transport in motor neurons causing late-onset progressive degeneration" NEURON, vol. 34, no. 5, 30 May 2002 (2002-05-30), pages 715-727

D9: TOKITO M K ET AL: "The genomic structure of DCTN1, a candidate gene for limb-girdle muscular dystrophy (LGMD2B)" BIOCHIMICA ET BIOPHYSICA ACTA . GENE STRUCTURE AND EXPRESSION, ELSEVIER, AMSTERDAM, NL, vol. 1442, no. 2-3, 8 November 1998 (1998-11-08), pages 432-436

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

2.1) The present set of claims refers to an extremely large number of possible cytoplasmic dynein heavy chain1 polypeptides 'modified by substitution, deletion or insertion of at least one amino acid compared to the wild type polypeptide' and having an 'altered biological activity compared to the wild type polypeptide' and genes encoding said polypeptides. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the claimed cytoplasmic dynein heavy chain1 polypeptides and genes encoding said polypeptides, as defined by SEQ ID NOs: 1-6 and 17-24.
Some of the claimed nucleic acid sequences in claims 122-185 refer to extremely large genomic fragments, comprising multiple open reading frames. Disclosure and support in the sense of Articles 5 and 6 PCT may only be found for the proteins and SEQ ID NOs listed in claim 132.

2.2) Claims 79-94 and 96-100 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item IV

Lack of unity of invention

3) The inventions of the present application are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, for the following reasons:

The common concept linking the inventions of the present application seems to be that the dynactin/dynein complex and proteins associated thereto are involved in motor neuron diseases. This concept was already known at the filing date of the present application, in particular D1 (figures 3, 5 and 7; page 724, right-handed column) indicates that overexpression of dynamitin, one of the components of the dynactin/dynein complex, results in late onset motor neuron disease.
The inventions are therefore not linked by a single general inventive concept.

This Authority therefore considers that there are 18 inventions covered by the claims indicated as follows:

Invention 1: polypeptide and polynucleotide sequences SEQ ID NOs:3-6, defining cytoplasmic dynein heavy chain1 polypeptides with a point mutation in the position corresponding to Y1055 of the murine wild type amino acid sequence defined by SEQ ID NO:2 (claims 27, 36, 42, 43 (all complete); 28-35, 37-41, 44, 46, 47, 50-52, 55-64, 66, 68-73, 75-81, 89-94, 99-121 (all partially)).
Invention 2: the cloning and sequencing of human cytoplasmic dynein heavy chain1 and the resulting nucleic acid and amino acid sequences, defined by SEQ ID NOs:17 and 18 (claims 48, 65 (all complete); 39-47, 50-52, 55-60, 63, 64, 66-75, 78-81, 96, 99-107, 114-121, 132-151, 160, 162-175, 177-185 (all partially as far as applicable)).
Invention 3: polypeptide and polynucleotide sequences SEQ ID NOs:19-24, defining exon 13 or exons 12+13 of cytoplasmic dynein heavy chain1 gene and the corresponding polypeptides (claim 49 (complete); 44, 45, 50, 63, 64, 67-77, 81, 97-100 (all partially)).
Inventions 4-18: provision of a method of identifying mutations in nucleic acid sequences encoding polypeptides interacting with the dynactin/dynein complex, whereby invention 4 relates to cytoplasmic dynein intermediate chain defined by SEQ ID NOs:30-33, 61, 62, 64, 65, 70-170; invention 5 relates to cytoplasmic dynein light intermediate chain defined by SEQ ID NOs:34-37; invention 6 relates to cytoplasmic dynein 10kD light chain defined by SEQ ID NOs:38, 39; invention 7 relates to Tctex defined by SEQ ID NOs:40, 41; invention 8 relates to cytoplasmic dynein light chain 2B defined by SEQ ID NO:42; invention 9 relates to DCTN1 defined by SEQ ID NOs:43, 44, 67, 68, 108-112, 114-158;

invention 10 relates to DCTN2 defined by SEQ ID NOs:159 or 45; invention 11 relates to DCTN3 defined by SEQ ID NOs:46 or 47; invention 12 relates to DCTN4 defined by SEQ ID NOs:48 or 49; invention 13 relates to DCTN5 defined by SEQ ID NOs:50; invention 14 relates to DCTN6 defined by SEQ ID NOs: 51 or 52; invention 15 relates to ARP1 defined by SEQ ID NOs: 53 or 54; invention 16 relates to ARP11 defined by SEQ ID NOs: 55 or 56; invention 17 relates to HAP1 defined by SEQ ID NOs: 57 or 58; invention 18 relates to CLIP-170 defined by SEQ ID NOs:59 or 60 (claims 132-151, 160, 162-175, 177-185, all in part as far as applicable).

An International Search Report has been drawn up for all 18 inventions.
For practical reasons, an opinion is given on all inventions and the lack of unity objection will be dealt with in the regional phase.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

INVENTION 1

NOVELTY (Article 33(2) PCT)

4.1) The prior art does not disclose a dynein cytoplasmic heavy chain 1 protein, with a point mutation as defined by the amino acid sequences of SEQ ID NOs:4 and 6, encoded by nucleic acid sequences defined by SEQ ID NOs:3 and 5 .

D1 and **D2** disclose antibodies that recognise cytoplasmic dynein heavy chain1 and are considered to be able to react with the modified dynein cytoplasmic heavy chain1 polypeptide of the present application, since there is only one amino acid different between the wild type and modified polypeptide. Therefore subject-matter relating to antibodies against the polypeptides defined by SEQ ID NOs:4 and 6 lacks novelty.

D3 discloses antisense oligonucleotide to inhibit expression of human cytoplasmic dynein heavy chain1 in melanocytes (D3, page 992, left-handed column, paragraph 3; figure 7), said oligonucleotides are considered to be complementary to sequences within human and mouse cytoplasmic dynein heavy chain1 defined by SEQ ID NOs:2, 4, 6 and 18.

D4 discloses a ENU mutagenesis screen for mouse mutants and has identified 4 strains displaying a similar phenotype of the cra1 mouse of the present invention (D4, table 1). In view of a later scientific publication by the applicant (**D5**), indicating the relationship between the screen of D4 and the cra1 model of the present application, it is considered that the cra1 mutant of the present invention is **identical** to the mutant derived from the ENU screen disclosed in D4, therefore the subject-matter relating to a transgenic non-human animal comprising the modified cytoplasmic dynein heavy chain1 polypeptide defined by SEQ ID NOs:4 or 6 is not novel.

INVENTIVE STEP (Article 33(3) PCT)

4.2) Since (partial) sequences encoding murine (**D6**) and human (**D7**) cytoplasmic dynein heavy chain1 polypeptides were known in the art at the priority date of the present application, and since the nucleic acids of the present invention only differ in one codon, the design of ribozymes specific for said nucleic acids is considered to be routine practise for the skilled person.

INVENTION 2

NOVELTY (Article 33(2) PCT)

5.1) The full length sequence encoding human cytoplasmic dynein heavy chain1 polypeptide is not disclosed in the prior art.

INVENTIVE STEP (Article 33(3) PCT)

5.2) Sequences encoding full length murine (**D6**) and partial human (**D7**, KIAA325 in table 2) cytoplasmic dynein heavy chain1 polypeptides were known in the art at the priority date of the present application, therefore the provision of the full length human sequence and applications thereof using standard molecular biological techniques does not involve inventive activity of the skilled person.

INVENTION 3

NOVELTY (Article 33(2) PCT)

6.1) Nucleic acid sequences comprising the sequence encoding exon 12 and/or 13 of dynein heavy chain1 polypeptide are known from **D3** (fig. 2) and polypeptides **comprising** exons 12 and/or 13 have been used in to identify the homodimerization domain of cytoplasmic dynein heavy chain1 polypeptide (**D2**, fig. 9, 10).

INVENTIVE STEP (Article 33(3) PCT)

6.2) It is not suggested in the prior art to use the polypeptides **consisting of** exon 12 and/or 13 for diagnosis or therapy. However, the present application does not demonstrate a technical effect of selection of these parts of the cytoplasmic dynein heavy chain1 polypeptide, hence no inventive step may be acknowledged.

INVENTIONS 1-3:

INDUSTRIAL APPLICABILITY (Article 33(4) PCT)

7.1) For the assessment of the present claims 79-94 and 96-100 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

ARTICLES 5 AND 6 PCT

7.2) The use of (modified) dynein heavy chain1 polypeptides for diagnostic or therapeutic applications is not disclosed and supported in the sense of Articles 5 and 6 PCT.

INVENTIONS 4-18

NOVELTY (Article 33(2) PCT)

8.1) The prior art does not disclose a method of identifying mutations in the claimed subunits of the dynactin/dynein complex as a marker indicative for a neurodegenerative disease.

INVENTIVE STEP (Article 33(2) PCT)

8.2) **D8** discloses that aberrant expression of dynamitin, one of the components of the dynactin/dynein complex, renders dynactin nonfunctional, resulting in the development of motor neuron degenerative disease. **D9** indicates that mutations of the drosophila $p150^{glued}$ subunit is related to defective neuronal development (D9, page 435, left-hand column, last paragraph).

The present application suggests to identify mutations in other subunits of the dynactin/dynein complex as marker for neurodegenerative disease(s), but does not demonstrate an effect of the selection of the further subunits of the dynactin/dynein

complex to identify markers for neurodegenerative diseases. Therefore no inventive step may be acknowledged for the subject-matter of claims 122-185.

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